

**WHAT IS CLAIMED:**

1. A protein of mammalian origin having a molecular weight of about 125  
5 kDa which binds to a GST-FKBP-Rapamycin complex.
2. A protein of Claim 1 which is of human origin.
3. A recombinantly produced protein of mammalian origin having a  
10 molecular weight of about 125 kDa which binds to a GST-FKBP-Rapamycin complex.
4. A recombinantly produced protein comprising a partial amino acid  
sequence of the protein of Claim 3.
- 15 5. cDNA of mammalian origin which encodes a 125 kDa protein which  
binds to a GST-FKBP-Rapamycin.
6. DNA encoding for a protein of mammalian origin having a molecular  
weight of about 125 kDa which binds to a GST-FKBP-Rapamycin complex.  
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7. Antisense RNA derived from a cDNA clone, the cDNA clone encoding  
for a protein of mammalian origin having a molecular weight of about 125 kDa which  
binds to a GST-FKBP-Rapamycin complex.
- 25 8. Antisense DNA derived from a cDNA clone, the cDNA clone encoding  
for a protein of mammalian origin having a molecular weight of about 125 kDa which  
binds to a GST-FKBP-Rapamycin complex.
9. A protein of mammalian origin having a molecular weight of about 148  
30 kDa which binds to a GST-FKBP-Rapamycin complex.
10. A protein of Claim 9 which is of human origin.

11. A recombinantly produced protein of mammalian origin having a molecular weight of about 148 kDa which binds to a GST-FKBP-Rapamycin complex.
12. A recombinantly produced protein comprising a partial amino acid  
5 sequence of the protein of Claim 11.
13. cDNA of mammalian origin which encodes a 148 kDa protein which binds to a GST-FKBP-Rapamycin.
14. DNA encoding for a protein of mammalian origin having a molecular  
10 weight of about 148 kDa which binds to a GST-FKBP-Rapamycin complex.
15. Antisense RNA derived from a cDNA clone, the cDNA clone encoding  
15 for a protein of mammalian origin having a molecular weight of about 148 kDa which binds to a GST-FKBP-Rapamycin complex.
16. Antisense DNA derived from a cDNA clone, the cDNA clone encoding  
20 for a protein of mammalian origin having a molecular weight of about 148 kDa which binds to a GST-FKBP-Rapamycin complex.
17. A protein of mammalian origin having a molecular weight of about 208  
kDa which binds to a GST-FKBP-Rapamycin complex.
18. A protein of Claim 17 which is of human origin.  
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19. A recombinantly produced protein of mammalian origin having a  
molecular weight of about 208 kDa which binds to a GST-FKBP-Rapamycin complex.
20. A recombinantly produced protein comprising a partial amino acid  
30 sequence of the protein of Claim 19.
21. cDNA of mammalian origin which encodes a 208 kDa protein which binds to a GST-FKBP-Rapamycin.

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22. DNA encoding for a protein of mammalian origin having a molecular weight of about 208 kDa which binds to a GST-FKBP-Rapamycin complex.

5 23. Antisense RNA derived from a cDNA clone, the cDNA clone encoding for a protein of mammalian origin having a molecular weight of about 208 kDa which binds to a GST-FKBP-Rapamycin complex.

10 24. Antisense DNA derived from a cDNA clone, the cDNA clone encoding for a protein of mammalian origin having a molecular weight of about 208 kDa which binds to a GST-FKBP-Rapamycin complex.

25. A protein of mammalian origin having a molecular weight of about 210 kDa which binds to a GST-FKBP-Rapamycin complex.

15 26. A protein of Claim 25 which is of human origin.

27. A protein of Claim 25 which includes one or more of the following internal amino acid sequences:

- 20 a) ILLNIEHR;  
B) LIRPYMEPIIK;  
c) DXMEAQE; and  
25 d) QLDHPLPTVHPQVTYAYM(K)

30 28. A recombinantly produced protein of mammalian origin having a molecular weight of about 210 kDa which binds to a GST-FKBP-Rapamycin complex.

29. A recombinantly produced protein comprising a partial amino acid sequence of the protein of Claim 28.

35 30. A recombinantly produced protein of Claim 25 which includes one or more of the following internal amino acid sequences:

- a) ILLNIEHR;

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- B) LIRPYMEPIK;
- c) DXMEAE; and
- 5 d) QLDHPLPTVHPQVTYAYM(K)

31. A cDNA of mammalian origin which encodes a 210 kDa protein which binds to a GST-FKBP-Rapamycin.

- 10 32. A cDNA of Claim 31 which encodes a 210 kDa protein which includes one or more of the following internal amino acid sequences:

- a) ILLNIEHR;
- 15 B) LIRPYMEPIK;
- c) DXMEAE; and
- d) QLDHPLPTVHPQVTYAYM(K)

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33. DNA encoding for a protein of mammalian origin having a molecular weight of about 210 kDa which binds to a GST-FKBP-Rapamycin complex.

- 25 34. A DNA of Claim 33 in which the protein of mammalian origin includes one or more of the following internal amino acid sequences:

- a) ILLNIEHR;
- 30 B) LIRPYMEPIK;
- c) DXMEAE; and
- d) QLDHPLPTVHPQVTYAYM(K)

- 35 35. Antisense RNA derived from a cDNA clone, the cDNA clone encoding for a protein of mammalian origin having a molecular weight of about 210 kDa which binds to a GST-FKBP-Rapamycin complex.

- 40 36. An antisense RNA derived from a cDNA clone of Claim 35 the cDNA clone encoding for a protein of mammalian origin which includes one or more of the following internal amino acid sequences:

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- a) ILLNIEHR;
- B) LIRPYMEPIK;
- c) DXMEAQE; and
- d) QLDHPLPTVHPQVTYAYM(K)

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10        37.     Antisense DNA derived from a cDNA clone, the cDNA clone encoding for a protein of mammalian origin having a molecular weight of about 210 kDa which binds to a GST-FKBP-Rapamycin complex.

15        38.     An antisense DNA derived from a cDNA clone of Claim 37 the cDNA clone encoding for a protein of mammalian origin which includes one or more of the following internal amino acid sequences:

- a) ILLNIEHR;
- 20        B) LIRPYMEPIK;
- c) DXMEAQE; and
- d) QLDHPLPTVHPQVTYAYM(K)

25        39.     A process for isolating a protein having a molecular weight of about 125 kDa, about 148 kDa, about 208 kDa, or about 210 kDa which binds to a GST-FKBP-Rapamycin complex from mammalian cells, the process comprising:

- 30                a)     lysing the mammalian cells in the presence of a buffering agent, a low salt, a chelating agent, a protease inhibitor, and a reducing agent at a temperature which minimizes protein degradation, the lysing creating unbroken cells, cell nuclei, and lysates, the lysates including cellular membrane fractions and cellular debris;
- 35                b)     preclearing unbroken cells and cell nuclei from the lysates at a temperature which minimizes protein degradation to create a precleared lysate;
- c)     concentrating the cellular membrane fractions of the mammalian cells from the precleared lysate, the membrane fractions containing membrane proteins;

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d) solubilizing the membrane proteins in a buffer containing a detergent which solubilizes the proteins, without detrimentally denaturing the proteins, at a temperature which minimizes protein degradation, resulting in solubilized proteins and mammalian cellular debris;

e) separating the solubilized proteins from the mammalian cellular debris;

10 f) incubating a solution containing the solubilized proteins in a buffer, the buffer containing a buffering agent, a salt, a reducing agent, one or more protease inhibitor(s), divalent cations, with an affinity resin to absorb to the affinity resin those proteins which have an binding affinity to the affinity resin at a temperature which allows binding to the affinity resin and minimizes protein degradation;

15 g) separating the affinity resin from the solution of step (f) at a temperature which minimizes protein degradation, yielding a solution (g) containing solubilized proteins which do not bind to the affinity resin in step (f);

20 h) incubating the solution (g) with Rapamycin or a Rapamycin analog ( $IC_{50}$  in LAF  $< 500$ nM) complexed to a fusion protein of FKBP12+protein, the fusion protein enhancing the isolation of the desired about 125 kDa protein, about 148 kDa, about 208 kDa, or about 210 kDa yielding a mixture (h) containing the desired proteins having a molecular weight of about 125 kDa, about 148 kDa, about 208 kDa, or about 210 kDa bound to a fusion FKBP protein :Rapamycin complexes or fusion FKBP protein :Rapamycin analog complexes;

30 i) incubating the mixture (h) containing the desired proteins having a molecular weight of about 125 kDa, about 148 kDa, about 208 kDa, or about 210 kDa bound to fusion FKBP protein :Rapamycin complexes or fusion FKBP protein :Rapamycin analog complexes with an affinity resin which binds to the fusion protein at a temperature and for a time which allows the binding to the affinity resin and minimizes protein degradation, bound to a fusion FKBP protein :Rapamycin complexes or fusion FKBP protein :Rapamycin analog complexes;

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j) rinsing the affinity resin (i) containing the bound complexes with a buffer which dissociates binding of proteins other than the desired about 125 kDa, about 148 kDa, about 208 kDa, or about 210 kDa proteins;

k) eluting the about 125 kDa, about 148 kDa, about 208 kDa, or about 210 kDa protein: fusion FKBP protein:rapamycin complexes fusion or the 125 kDa protein:FKBP protein:rapamycin analog complexes from the affinity resin (j) with a buffer;

l) separating the proteins eluted in step (k) by size.

40. A method for identifying an immunomodulatory or anti-tumor agent, comprising the steps of:

a) combining a substance to be tested with a mammalian protein having a molecular weight of about 125 kDa, about 148 kDa, about 208 kDa, or about 210 kDa which binds to an FKBP-Rapamycin complex, the protein being bound to a solid support:

b) maintaining the substance to be tested and the protein bound to the solid support of step (a) under conditions appropriate for binding of the substance to be tested with the protein, and

c) determining whether binding of the substance to be tested occurred in step (b).

41. A method of Claim 40 in which the mammalian protein includes one or more of the following internal amino acid sequences:

a) ILLNIEHR;

B) LIRPYMEPIK;

c) DXMEAQE; and

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d) QLDHPLPTVHPQVTYAYM(K)

5 42. A method for identifying an immunomodulatory or anti-tumor agent, comprising the steps of:

a) combining a substance to be tested with a mammalian protein having a molecular weight of about 125 kDa, about 148 kDa, about 208 kDa, or about 210 kDa which binds to an FKBP-Rapamycin complex, the protein being bound to a solid support:

b) maintaining the substance to be tested and the protein bound to the solid support of step (a) under conditions appropriate for binding of the substance to be tested with the protein, and

c) determining whether the presence of the substance to be tested modulated the activity of the mammalian protein having a molecular weight of about 125 kDa which binds to an FKBP-Rapamycin complex.

20 43. A method of Claim 42 in which the mammalian protein includes one or more of the following internal amino acid sequences:

a) ILLNIEHR;

25 B) LIRPYMEPIK;

c) DXMEAQE; and

d) QLDHPLPTVHPQVTYAYM(K)

30 44. A method for detecting, in a biological sample, rapamycin, rapamycin analogs or rapamycin metabolites which, when complexed with a FKBP, bind to a mammalian protein having a molecular weight of about 125 kDa, about 148 kDa, about 208 kDa, or about 210 kDa which binds to a GST-FKBP-Rapamycin complex, the method comprising the steps of:

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a) combining the biological sample with a FKBP to form a first mixture containing, if rapamycin, rapamycin analogs or rapamycin metabolites are present in the biological sample, a rapamycin:FKBP complexes, rapamycin analog:FKBP complexes, or rapamycin metabolite:FKBP complexes;

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b) creating a second mixture by adding the first mixture to a protein having a molecular weight of about 125 kDa, about 148 kDa, about 208 kDa, or about 210 kDa which binds to a GST-FKBP-Rapamycin complex, the protein having a molecular weight of about 125 kDa being bound to a solid support ;

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c) maintaining the second mixture of step (b) under conditions appropriate for binding the rapamycin:FKBP complexes, rapamycin analog:FKBP complexes, or rapamycin metabolite:FKBP complexes, if present, to the protein having a molecular weight of about 125 kDa, about 148 kDa, about 208 kDa, or about 210 kDa which binds to a GST-FKBP-Rapamycin complex; and

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d) determining whether binding of the rapamycin:FKBP complexes, rapamycin analog:FKBP complexes, or rapamycin metabolite:FKBP complexes and the protein occurred in step (c).

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45. A method of Claim 44 in which the protein includes one or more of the following internal amino acid sequences:

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a) ILLNIEHR;

B) LIRPYMEPIK;

c) DXMEAQE; and

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d) QLDHPLPTVHPQVTYAYM(K)

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46. A method for modulating the immune system of a mammal in need thereof, the method comprising administering to the mammal an immunomodulatory amount of antisense RNA derived from a cDNA clone which encodes for a protein having a molecular weight of about 125 kDa, about 148 kDa, about 208 kDa, or about 210 kDa which binds to an FKBP-Rapamycin complex.

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47. A method of Claim 46 in which the protein includes one or more of the following internal amino acid sequences:

- 5 a) ILLNIEHR;
- B) LIRPYMEPIK;
- 10 c) DXMEAE; and
- d) QLDHPLPTVHPQVTYAYM(K)

48. A method for modulating the immune system of a mammal in need thereof, the method comprising administering to the mammal an immunomodulatory  
15 amount of antisense DNA derived from a cDNA clone which encodes for a protein having a molecular weight of about 125 kDa, about 148 kDa, about 208 kDa, or about 210 kDa which binds to an FKBP-Rapamycin complex.

49. A method of Claim 48 in which the protein includes one or more of the following internal amino acid sequences:

- 20 a) ILLNIEHR;
- B) LIRPYMEPIK;
- 25 c) DXMEAE; and
- d) QLDHPLPTVHPQVTYAYM(K)

50. A protein of mammalian origin which includes one or more of the following internal amino acid sequences:

- 30 a) ILLNIEHR;
- 35 B) LIRPYMEPIK;
- c) DXMEAE; and
- 40 d) QLDHPLPTVHPQVTYAYM(K)

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51. A recombinantly produced protein which includes one or more of the following internal amino acid sequences:

- 5 a) ILLNIEHR;
- B) LIRPYMEPILK;
- c) DXMEAE; and
- 10 d) QLDHPLPTVHPQVTYAYM(K)

52. A cDNA which encodes a protein which includes one or more of the following internal amino acid sequences:

- 15 a) ILLNIEHR;
- B) LIRPYMEPILK;
- 20 c) DXMEAE; and
- d) QLDHPLPTVHPQVTYAYM(K)

53. A DNA encoding for a protein of mammalian origin which includes one or more of the following internal amino acid sequences:

- 25 a) ILLNIEHR;
- 30 B) LIRPYMEPILK;
- c) DXMEAE; and
- 35 d) QLDHPLPTVHPQVTYAYM(K)

54. An antisense RNA derived from a cDNA clone encoding for a protein of mammalian origin which includes one or more of the following internal amino acid sequences:

- 40 a) ILLNIEHR;
- B) LIRPYMEPILK;
- 45 c) DXMEAE; and
- d) QLDHPLPTVHPQVTYAYM(K)

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55. An antisense DNA derived from a cDNA clone encoding for a protein of mammalian origin which includes one or more of the following internal amino acid sequences:

- 5       a) ILLNTEHR;  
          B) LIRPYMEPIIK;  
10       c) DXMEAQE; and  
          d) QLDHPLPTVHPQVTYAYM(K)